PhD thesis

Mechanism of action and neuroprotective potential of estradiol and new non-genomic estrogen-like signaling activators

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1. Introduction

1.1. Estrogens

Female sexual hormones, including estrogens, were discovered, and characterized in the 1930s. Almost 100 years have passed since the first breakthrough, the interest in estrogen, reflected by high number of publications, is still increasing.

In humans there are three important estrogen molecules: estrone (E1), 17β-estradiol (E2), and estriol (E3). E2 is the most potent estrogen hormone produced in the ovary. Even though estrogen hormones affect the function of almost all genes in the mammalian body, they are best known for their major role in reproduction. Estrogens modulate the development of secondary female characteristics, and mediate reproduction, menstrual cycle, sexual behavior, and emotional background. During menopause or after surgical removal of the ovaries, the concentration of E2 decreases, resulting in unpleasant symptoms like hot flushes, emotional disturbances, memory loss, and even osteoporosis (Nelson, 2008). Indeed, E2 helps to develop long bones and pubic epiphyses during puberty. Moreover, it protects the bone structure by inhibiting the degrading osteoclast activity. With these mechanisms it exerts positive effects on osteoporosis in both estrogen-deficient and post-menopausal women (Levin et al., 2018). The treatment of menopausal symptoms was one of the very first therapeutic applications of estrogens. Nevertheless, E2 may affect many other physiological processes and plays an important role in the development, thereby also in the treatment of various diseases.

1.2. Mechanism of action

1.2.1. Classical estradiol pathway

According to the classical view E2, as a lipophilic molecule passes through the cell membrane by passive diffusion, and in the cytoplasm binds to intracellular estrogen receptors (ERs). The biologically active ligand–receptor complex induces a complicated sequence of events that begins with conformational changes in the receptor, followed by receptor dimerization, and leads to direct translocation of the activated ligand–receptor complex to the nucleus (Nilsson et al., 2001). The dimerized receptor complex interacts with nuclear receptor coactivators, such as the 160-kDa steroid receptor coactivator protein (SRC/P160) or the cyclic adenosine 3′, 5′-monophosphate (cAMP)-responsive element-binding protein (CREB) (Le Dily & Beato, 2018). The entire complex then binds to a specific DNA sequence, the estrogen responsive element (ERE), to stimulate transcription, which ultimately leads to the synthesis of new proteins (Ayaz et al., 2019). Cytonuclear ERs are key receptors in activating the classical

pathway. However, the classical mechanism of action alone is not sufficient to fully elucidate the broad spectrum of estrogenic effects.

1.2.2. Non-classical estradiol pathway

The non-classical mechanism of E2 is mediated by direct effects on ion channels or by the activation of intracellular signaling via protein kinase A (PKA), phosphatidylinositol-3-OH kinase (PI3K), and mitogen-activated protein kinase (MAPK) pathways (Bryant et al., 2005). E2 can directly bind to membrane receptors and rapidly activates intracellular signaling pathways, leading to ERE-independent indirect gene transcription (McDevitt et al., 2008). In addition to initiating transcription of non-ERE-dependent genes, another key feature of the non-classical pathway is the speed of response. An early study by Szego and Davis showed a rapid increase in cAMP levels in uterine tissue 15s after E2 administration (Szego & Davis, 1967). In addition, E2 has been reported to activate the MAPK pathway in seconds, through a phosphorylation cascade on mitogen-activated protein kinase kinase (MEKKs) and MAPK, the extracellular regulated kinase 1/2 (ERK1/2) will modulate the transcription of genes (Chang et al., 2003). An alternative pathway exists via the PI3K signaling molecules that can be activated by ERs as well as by GPERs (Barabas et al., 2006) and interact with protein kinase B (Akt), resulting in the activation of endothelial nitric oxide synthase (eNOS) (Sirohi et al., 2022).

1.3. 17β-estradiol in neuroprotection

The higher prevalence of dementia in postmenopausal women suggests that female sexual steroids, especially E2, might be preventive. Indeed, there are significant amounts of ERs in the centers of episodic and working memory, the hippocampus (HC) and prefrontal cortex (PFC), so during perimenopause, the E2 fluctuation can cause a significant cognitive deficit (Genazzani et al., 2007). Alongside memory formation, E2 also influences neuroinflammation. Unstable E2 levels in the brain were found to be associated with the development of neurogenic inflammation leading to migraine (Karkhaneh et al., 2015).

1.3.1. Estradiol and basal forebrain cholinergic neurons

The cholinergic system is one of the most important and extensive neurotransmitter networks in the CNS. Most cholinergic neurons can be found in the basal forebrain (BF). The BF consists of four anatomical regions: the medial septum (MS), Broca's ventral and horizontal diagonal bands (vDB and hDB) and the substantia innominata and nucleus basalis magnocellularis complex (SI/NBM; this neuroanatomical structure is called Meynert nucleus in the human brain) (Hampel et al., 2018). E2 can influence the morphology, neurochemical and electrophysiological properties of basal forebrain cholinergic (BFC) neurons. Following

ovariectomy (OVX), E2 treatment can increase the size of the cholinergic neurons in the hDB and SI/NBM, and the length and degree of branching of these neurons (Ping et al., 2008). Gibbs's research group also showed that application of E2 increases acetylcholine (ACh) synthesis in BFC neurons by increasing cholin-acetyltransferase (ChAT) enzyme expression and activity (Gibbs & Aggarwal, 1998). *In vivo* experiments in rats showed that E2 restored the synaptic integrity of the cholinergic system in the cortex following cholinergic cell death induced by NMDA or $A\beta_{1\text{-}42}$ injection into the SI/NBM (Kwakowsky et al., 2016). Although the neuroprotective effects of E2 on BFC neurons are crucial, the mechanism is still unclear. The molecular basis of this effect may be the known ERα expression of BFC neurons (Kalesnykas et al., 2005).

1.3.2. Relation between E2 and Alzheimer's disorder

Alzheimer's disorder (AD) is the most common type of dementia, which is among the top 10 leading causes of death in the world. It is characterized by disturbances of memory, attention, and sleep. The patients often have difficulties in their daily life due to their impaired behavioral abilities (Scheltens et al., 2016). Morphologically, Aβ plaques and pTau aggregates appear in the HC, cortex and amygdala, brain areas that are critical in cognitive and emotional function (Querfurth & LaFerla, 2010). Based upon these morphological changes the most prevalent animal model of AD was Aβ injection into the brain (either intracerebroventricullarly, or in the HC or other areas) (Facchinetti et al., 2018). However, due to the lack of effectiveness of Aβ elimination newer models, mostly transgenic mice lines became more popular.

The three major risk factors of AD are age, gender and genetical mutations (Nebel et al., 2018). It is well known that the incidence of AD is increasing with age, but it is also important to note that women represent 70% of the patients (Fisher et al., 2018). The increasing female prevalence among elderly can be due to hormonal change during menopause (Pike, 2017). Indeed, both E2 and PG play pivotal role in neuroprotection, thereby their administration improves cognitive function, memory, attention, synaptic plasticity, spine density and dendrite formation (Kwakowsky et al., 2016). Consequently, the loss of the ovarian hormones can affect these functions, and increase the appearance of amyloidogenic markers, aggravating the progression of AD.

1.4. The Problem with E2 and Hormone Replacement Therapy (HRT)

Nowadays, E2 therapy might be used as hormonal contraception, as HRT during menopause, or as feminizing hormone therapy to treat gender dysphoria in transgender women. Indications

for the use of HRT in the postmenopausal age have risen sharply since the late 1980s, claiming that “menopause is a hormone deficiency disease, curable and totally preventable, just take estrogen” (Wilson, 1966), as several epidemiological studies have shown that the use of HRT reduces the risk of osteoporosis, coronary heart disease, and all-cause mortality (Lobo et al., 2016). However, large-scale, controlled clinical trials in the late 1990s have resulted in a reassessment of the favorable paradigm of HRT. In 1998, the “Heart and Estrogen/progestin Replacement Study (HERS)” showed that HRT did not reduce the risk of recurrent myocardial infarction (Hulley et al., 1998). Subsequently, in 2002 and 2004, the Woman Health Initiatives (WHI) clinical studies showed that neither the E2-PG combination, nor E2 alone reduced the risk of coronary heart disease (Anderson et al., 2004; Shumaker et al., 2004). Moreover, the combined HRT even increased the risk of stroke, venous thromboembolism, and breast cancer. Therefore, HRT is no longer recommended for prevention of either coronary artery disease or osteoporosis, and the beneficial effects are neglected due to these risks and severe side effects.

The serious side effects of HRT steered estrogen research into a different direction. The common goal was to find new formulas or molecules that do not have the harmful side effects of estrogen but have a positive effect on menopausal symptoms, including nervous system problems, vascular lesions, and osteoporosis.

1.5. Possible New Therapies: Activators of non-genomic estrogen-like signaling (ANGELS)

Several estrogen-like compounds are available with wide therapeutic indications divergently influencing different estrogen-related pathways: selective estrogen-receptor modulators (SERMs), phytoestrogens, selective estrogen-receptor downregulators or degraders (SERDs), GPER1 agonists/antagonists or aromatase inhibitors.

Activators of non-genomic estrogen-like signaling (ANGELS) are a new approach in E2 therapy. ANGELS can selectively activate the non-classical action of E2 (Manolagas et al., 2002). There are three known molecules with such effect: estren (4-estren-3alpha, 17beta-diol), compound A (2-(4-hydroxyphenyl)-3-methylbenzo[b]thiophen-5-ol), and compound B (3′,15b-dihydrocyclopropa [14,15]estra-1,3,5(10),8-tetraene-3,17alpha-diol), but neither of them are used in therapy, yet (Kwakowsky et al., 2013). Estren has bone-protective effects without influencing endometrial proliferation (Otto et al., 2008). It was shown to have neuroprotective potentials on BFC neurons in an animal model of AD (Kwakowsky et al., 2016). Moreover, estren also induces vasodilation and has vasoprotective effects.

Besides their therapeutic potential, novel HRTs and potential therapeutic agents help us to better understand the details of physiological E2 action. The perfect therapy with neuroprotective, antithrombotic, osteoprotective, and anticarcinogenic effects is yet to be discovered, but accumulating data might bring us closer to it.

2. Aims

The major aim of this PhD thesis was to identify new ANGELS molecules and confirm their non-classical mechanism of action and their neuroprotective properties in comparison to E2.

An initial step was an *in silico* selection of possible molecules, which was done in collaboration with Csaba Hetényi and Norbert Jeszenői, from the Department of Pharmacology and Pharmacotherapy based upon an available database. Then we synthesized the selected 15 molecules.

During my PhD the specific aims were:

1. To identify new ANGELS molecules without classical mechanism of action from the synthesized 15.
    1.1. To exclude *in vitro* the E2-like ERE activating potential of the molecules in MCF7 cells using a luciferase-based assay (Experiment 1.).
    1.2. To exclude further molecules with *in vivo* E2-like uterotropic side-effects (Experiment 2.).
2. To confirm *in vitro* that the selected ANGELS molecules have non-classical mechanism of action using MCF7 cells and Western blotting technique investigating the phosphorylation of downstream messenger molecules (ERK1/2, AKT and CREB) (Experiment 3.).

Based upon these steps the most promising ANGELS molecule (A3270) was selected and used in the upcoming experiments.

3. To determine the *in vivo* neuroprotective potential of the selected ANGELS on SI/NBM cholinergic neurons in a neurotoxic model of AD both in male and female mice in comparison to E2 using AChE and ChAT immunohistochemistry (Experiment 4.).
4. To investigate the *in vivo* neuroprotective effect of an acute E2 or ANGELS treatment on morphological (AChE, ChAT, Aβ and pTau) and behavioral (forced swim test, y-maze) alteration of a genetic model of AD (Experiment 5.).

5. To provide an animal model for further studies that incorporate menopause and easier to handle (no need to wait for it to grow old) we investigated whether OVX accelerates the appearance of bodily (fat accumulation by magnetic resonance imaging (MRI)), morphological (AChE, ChAT and Aβ) and behavioral changes (e.g. Y-maze, Morris water maze, fox odor test) in a genetic animal model of AD (3xTg-AD) (Experiment 6.).

3. Results and discussion

In the experiments presented here we identified 6 ANGELS compound without *in vivo* and *in vitro* classical effects and with *in vitro* activation of alternative E2 signalling pathways. The one selected compound with the best profile showed neuroprotective potential both in a neurotoxic and a transgenic model of AD already after a single treatment, however, at the given timepoint (24h prior testing) was not able to alter behaviour. Furthermore, we confirmed that OVX might accelerate the appearance of morphological alterations in the 3xTg-AD model, allowing earlier examinations, thus, shortening further drug development.

3.1. Classical mechanism of action (experiment 1,2)

E2 has a high affinity to the ERE transcriptional factor and this binding is knowingly the most important step in the classical mechanism of E2 (Koszegi & Cheong, 2022). Since HRT has many side effects, like increased risk of stroke, breast, and endometrial cancer, which can be mostly connected to their classical action, during new drug development excluding molecules first by their classical mechanism of action is essential. However, the relation between increased ERE activity and the presence of side-effects is not yet proved, thus, important to investigate. The presented positive and significant correlation between *in vitro* increased ERE activity and *in vivo* elevated uterus weight and epithelium layer thickness can prove the strong relation between the two parameters. Hence, our assay seems to be appropriate for the preselection of molecules, and to the identification of compounds that do not exert classical mechanism of action.

After the *in silico* computational docking, 15 ANGELS molecules were selected and synthesized, then tested for classical side-effects. Six did not induce ERE activation *in vitro*, which was in correlation with their *in vivo* uterotrophic potential. Thus, they were used in further experiments.

3.2. Non-classical mechanism of action (experiment 3)

E2 was able to induce non-classical actions after 5-minute treatment in an adenocarcinoma cell line (MCF-7), through the increased phosphorylation of AKT and ERK1/2. The 5 minutes

interval was key because non-classical actions are characterised also by their fast mechanism of action (Szego & Davis, 1967) After 10 minutes, the phosphorylation of these proteins started to decrease. Therefore, to test the quick ERK1/2 and AKT activating potential of the ANGELS molecules this 5-minute time point was used. All the tested 6 ANGELS molecules increased the phosphorylation of ERK1/2 and AKT in a dose identical to E2 (supraphysiological, therapeutic dose). The activation of the CREB protein was only seen after A3270 administration. The three investigated proteins have a pivotal role as second messengers in the MAPK-PKA-CREB pathway and were shown previously to be activated by E2 in different tissues, among others in the brain. For example, in the CA1 region of the hippocampus global ischemia promoted early dephosphorylation and inactivation of ERK1/2 and CREB, which was prevented by E2 treatment (Jover-Mengual et al., 2007). In the dentate gyrus sex difference was found in the phosphorylation status of these molecules. Intact female rodents had higher AKT pathway phosphoproteins than males, and OVX animals after E2 treatment showed an increase in MAPK and AKT pathways-related phosphoproteins (pERK1/2, pJNK, pAKT, and pGSK-3β) (Sheppard et al., 2022). The E2 activation of the MAPK pathway was also connected with cholinergic neurite outgrowth (Dominguez et al., 2004) and it was also proved that E2-mediated regulation of cholinergic expression in basal forebrain neurons also requires ERK1/2 activity (Pongrac et al., 2004). Regarding BFC neurons, Szego et. al showed that E2 treatment has direct, non-classical action in BFC neurons and activates the PKA and MAPK pathways via ER, leading to the phosphorylation of CREB in the SI/NBM cholinergic neurons *in vivo* (Szego et al., 2006). Later, the same research group published that the restorative effects of E2 on cholinergic fibers were blocked by the inhibition of MEK1/2 in the MAPK pathway and/or by the inhibition of PKA (Koszegi et al., 2011).

These tests were important to preselect the possible neuroprotective agents for further studies and to understand their exact non-classical mechanism of action.

3.3. Neuroprotection in a neurotoxic animal model of AD (experiment 4)

The $A\beta_{1-42}$ microinjection in the brain was widely used to model sporadic AD with the advantage to selectively examine the role of a specific brain area in the disease progress (Nichols et al., 2020). Previous studies from Ábrahám lab. showed that NMDA injection into the SI/NBM induced cholinergic cell loss locally and cholinergic fiber loss in the ipsilateral somatosensory cortex (SSC) (Koszegi et al., 2011). A single injection of E2 did not influence cholinergic cell body loss in the SI/NBM but increased the cholinergic fiber density in the SSC. These finding were later supplemented with an $A\beta_{1-42}$ injection model, where E2 had the same

effect. Moreover, not only E2, but another non-classical E2 pathway activator, estren, also restored the loss in cholinergic cortical projections and attenuated the $A\beta_{1-42}$-induced learning deficits (Kwakowsky et al., 2016). With the help of the same $A\beta_{1-42}$ injection model we confirmed that the one selected ANGELS (A3270) had similar effect as E2 and estren. The effect was not restricted to females but was detectable also in males suggesting a more general, sex-independent neuroprotective role of E2 as well as ANGELS. However, as there was no sex difference, we further concentrated on females only.

We must admit that this effect was more a prevention than treatment as it was applied 1h after neurotoxic intervention. Furthermore, the $A\beta_{1-42}$ neurotoxicity AD model has certain limitations, like not modelling the whole pathomechanism, requires surgical interventions and does not consider the genetical predispositions. Thus, to confirm curative potential, we used another model. Our research benefited from the advantages of the two (neurotoxic and genetical) AD models (Götz & Ittner, 2008).

3.4. Neuroprotection in a genetical model of AD (experiment 5)

We have chosen 3xTg-AD model since its development in 2003 (Oddo et al., 2003) it is one of the widely used preclinical model, thus, substantial data accumulated with its usage. Nevertheless, AD is more prevalent in females, thus, we concentrated on this sex. An earlier study showed that OVX in 3xTg-AD mice increased Aβ accumulation and worsened memory performance, while E2, but not PG, prevented these effects (Carroll et al., 2007).

In our hands, 48h after an acute E2 or ANGELS treatment no change was detected in the number of amyloid plaques in motor cortex (MC), SSC, basolateral amygdala (BLA) and dorsal-Subiculum of the hippocampus (dSub-HC), or in pTau tangles located in BLA, HC-CA3 or amygdalopiriform transition area (APir) of the brain. The lack of change can be due to the single treatment or the short/long time interval between the treatment and animal perfusion. The previously mentioned studies used chronic E2 treatment - for example implanted 90 days subcutaneous pellet containing 0.25 mg E2 (Carroll et al., 2007) - to investigate the Aβ plaques. Despite ineffectiveness on plaque formation both E2 and our ANGELS compound restored cholinergic fibre loss in the SSC without restoring the cell numbers in SI/NBM region even after a single administration. This was in line with their neuroprotective potential in the neurotoxic model. We used relatively young (6-month-old) animals; therefore, it is not surprising that short term memory deficit was not detectable on y-maze, not even after 2 weeks OVX as the symptoms supposed to appear later (Várkonyi et al., 2022). However, our previous studies repeatedly showed locomotor impairment in our model (Szabó et al., 2023; Várkonyi

et al., 2022), thus, the locomotor elevation after ANGELS treatment suggest some preventive role. E2 deeply influence emotions, and previous studies suggested that 24-48h after its administration depression-like behaviour in FST can be reduced (Estrada-Camarena et al., 2002). In line with this our E2 treatment increased struggling, the active escape movement, in the FST. However, our ANGELS compound had no such effect. As this molecule was specially selected for its non-classical, fast action, we might assume, that the studied time-point was not optimally chosen. Indeed, some studies suggested effectiveness of E2 already after 30 min (Rocha et al., 2005). However, our previous morphological results supported long-lasting influence, therefore we have chosen this timepoint.

All in all, the selected ANGELS had neuroprotective potential even in a transgenic animal model. However, we had to refine our model, as the requirement of old animals for AD studies did not allow high throughput examinations.

3.5. Effect of hormone depletion on the progression of AD (experiment 6)

In young, 4-month-old mice the 1-month long OVX exaggerated neurodegeneration (increased amyloid deposition in the BLA, and decreased cholinergic fibre density in the SSC), however, could not aggravate the appearance of AD-related symptoms in cognitive and anxiety-related behavioural tests.

We confirmed that our model worked, as OVX induced the expected increase in body weight with fat accumulation as well as decrease in uterus weight and lean body percentage. The lack of sexual steroids can cause an increased risk for obesity, since E2 and PG also mediate glucose and lipid metabolism, and affects adipocyte physiology (Mauvais-Jarvis, 2011). Importantly, obesity is a prominent risk factor for AD, by increasing Aβ plaques, adipokines, cytokines and effecting insulin homeostasis (Reviewed: (Picone et al., 2020)). The MRI data also showed a decreased body lean ratio in the OVX groups, which may be the prodrome of osteoporosis. Indeed, female sex hormone depletion was linked closely to low bone mineral density (Farkas et al., 2022).

According to the literature 3xTg-AD animals develop memory loss after 6-month (Belfiore et al., 2019). Hence our animals were between 4-5-months-old the results of the behavioural tests are not unexpected. However, we could not support our hypothesis, as the OVX did not aggravate the cognitive decline or emotional disturbances. The intact memory can also be explained by the lack of Aβ deposition in the hippocampus and cortical areas (Jameie et al., 2021).

Anxiety is a core symptom of postmenopausal women (Siegel & Mathews, 2015), as well as might be co-morbid with AD (Botto et al., 2022). An AD effect was visible in FOT measuring innate fear and anxiety-related behavior (Bruzsik et al., 2021). We found that 3xTg-AD animals spend more time freezing, which suggests that these animals were more frightened (Adhikari et al., 2015; Bruzsik et al., 2021). Also, 3xTg-AD animals spend less time exploring and rearing, which might reflect anxiety. Nevertheless, these findings may be related to the increased Aβ deposition in the BLA, as this region is responsible for formation of fear related responses and can be linked to anxious behavior (Adhikari et al., 2015). However, despite our expectation OVX had no effect and did not aggravate the symptoms.

Despite subtle behavioral changes, morphological differences were more equivocal. OVX was able to increase the number of amyloid plaques in the 3xTg-AD animals. However, we detected changes in the BLA, but not in the HC, SSC. We must note, that in much older animals OVX-induced Aβ formation was found also in the SSC and HC (Palm et al., 2014). This is also in line with human studies, where OXV patients were treated with HRT, which prevented Aβ deposition (Zeydan et al., 2019). OVX was also able to further aggravate AChE positive fiber loss seen in 3xTg-AD animals.

Nevertheless, OVX-induced acceleration in morphological sign makes this intervention suitable to promote studies in younger 3xTg-AD mice.

4. Conclusion

The *in silico* screening gave us the possibility to preselect molecules, which bind to ERs, and possibly have non-classical neuroprotective potentials. We confirmed that the *in vitro* ERE activity measurement can effectively identify molecules without classical E2-signaling activity in significant correlation with their classical uterotropic side-effect *in vivo*. Based upon these effects we were able to reduce the initial 15 molecule number to 6. Further *in vitro* tests confirmed the non-classical mechanism of action of these molecules as they activated (increased the phospho/non-phospho ratio) the MAPK-PKA pathway in MCF-7 cells after 5 minutes. One ANGELS molecule (cod name: A3270) was selected from the initial 15, which does not had increased ERE activity, uterotrophic side-effect, was able to activate not only AKT and ERK1/2, but also CREB proteins. The selected ANGELS showed neuroprotective potential in a neurotoxic model of AD, induced by $A\beta_{1\text{-}42}$ microinjection to the SI/NBM in both sexes, as well as in female transgenic mice model (3xTg-AD). Both E2 and the ANGELS

increased the fiber density of cholinergic neurons in the SSC but could not restore the cell loss of cholinergic neurons in the BFC. Single injection had no effect on studied behavior 24h later.

To promote further research lastly, we confirmed that OVX induced menopausal symptoms also in the 3xTg-AD mice and removal of the sexual steroids aggravated the appearance of AD-related morphological alterations in the brain without deep influence on behavior. Thus, the OVX in young, 3-month-old 3xTg-AD mice might be a suitable model for testing the effect of new treatment options at structural level, which can speed up testing (it is not necessary to wait 6-12 months for the animals to age).

5. List of publications

This dissertation is based on the following articles:

Farkas S, Szabó A, Hegyi AE, Török B, Fazekas CL, Ernszt D, Kovács T, Zelena D. (2022). *Estradiol and Estrogen-like Alternative Therapies in Use: The Importance of the Selective and Non-Classical Actions*, Biomedicines, 6;10(4):861. (IF= 4.75)

Farkas S, Szabó A, Török B, Sólyomvári C, Fazekas CL, Bánrévi K, Correia P, Chaves T, Zelena D. (2022). *Ovariectomy-induced hormone deprivation aggravates Aβ1-42 deposition in the basolateral amygdala and cholinergic fiber loss in the cortex but not cognitive behavioral symptoms in a triple transgenic mouse model of Alzheimer's disease.* Front Endocrinol (Lausanne) 11; 13:985424. (IF= 6.05)

Farkas S, Jeszenői N, Kövesdi E, Horváth I, Szabó A, Zelena D, Kovács T, Hetényi C, Ábrahám I (2023). *New estrogen-like compounds show selective non-classical mechanism of action in MCF-7 cells and neuroprotective potential in two mouse models of Alzheimer's disorder*. – under writing

Other publications:

Gáll Z, **Farkas S**, Albert Á, Ferencz E, Vancea S, Urkon M, Kolcsár M. (2020). *Effects of Chronic Cannabidiol Treatment in the Rat Chronic Unpredictable Mild Stress Model of Depression.* Biomolecules. 22;10(5):801. (IF= 4.57)

Várkonyi D, Török B, Sipos E, Fazekas CL, Bánrévi K, Correia P, Chaves T, **Farkas S**, Szabó A, Martínez-Bellver S, Hangya B, Zelena D. (2022). *Investigation of Anxiety- and Depressive-like Symptoms in 4- and 8-Month-Old Male Triple Transgenic Mouse Models of Alzheimer's Disease*. Int J Mol Sci. 2022 16;23(18):10816. (IF= 6.20)

Szabó A, **Farkas S**, Fazekas C, Correia P, Chaves T, Sipos E, Makkai B, Török B, Zelena D. (2023) *Temporal Appearance of Enhanced Innate Anxiety in Alzheimer Model Mice*. Biomedicines. 18;11(2):262. (IF= 4.75)

Kövesdi E, Udvarácz I, Kecskés A, Szőcs S, **Farkas S**, Faludi P, Jánosi TZ, Ábrahám IM, Kovács G. (2023). *17β-estradiol does not have a direct effect on the function of striatal cholinergic interneurons in adult mice in vitro.* Front Endocrinol (Lausanne). 4;13:993552. (IF= 6.05)

Total impact factor: 32.37

6. Other first author conference presentations

Farkas S, Szabó A, Török B, Fazekas CL, Bánrévi K, Correia P, Chaves T, Ábrahám IM, Kovács T and Zelena D: *Chronic treatment with estrogen-like compounds shows anxiolytic and neuroprotective potential in a triple transgenic mouse model of Alzheimer's disorder,* FENS regional meeting 2023 (FRM2023), Poster, Portugal, Algarve, 2023.05.3-5.

Farkas S, Szabó A, Kövesdi E, Faludi P, Ábrahám IM, Kovács T and Zelena D: *New estrogen-like non-classical mechanism activators show neuroprotective properties in two mouse models of Alzheimer's disorder,* European Neuroscience Conference by Doctoral Students (ENCODS), Poster, Portugal, Faro, 2023.05.1-2.

Farkas S, Szabó A, Kövesdi E, Faludi P, Ábrahám IM, Kovács T and Zelena D: *Estrogen-like compounds show selective non-classical mechanism of action in MCF-7 cells and neuroprotective potential in two mouse models of Alzheimer's disorder,* IV. Centre for Neuroscience PhD and TDK conference, Talk, First Place, Hungary, Pécs, 2023.03.31-04.01.

Farkas S, Szabó A, Jasper V, Nyers-Marosi K, Petrovai B, Ábrahám IM, Kovács T and Zelena D: *Quadruple-transgenic mice model of Alzheimer's disorder, with Aβ1-42 and pTau deposition, and cholinergic neuron specific Cre expression,* International Neuroscience Meeting- IBRO, ANA Workshop, Poster, Hungary, Budapest 2023.02.01-03.

Farkas S, Szabó A, Jasper V, Nyers-Marosi K, Petrovai B, Ábrahám IM, Kovács T and Zelena D: *Quadruple-transgenic mice model of Alzheimer's disorder, with Aβ1-42 and pTau deposition, and cholinergic neuron specific Cre expression*, 6th Hungarian Neuroscience Doctoral Conference for Undergraduate Students, Graduate Students and Junior Postdocs, Poster, Hungary, Budapest, 2023.01.31.

Farkas S, Szabó A, Kovács T, Ábrahám IM and Zelena D: *Acute treatment with an estrogen-like compound shows neuroprotective potential and changes the behavior of a triple transgenic mouse model of Alzheimer's disorder,* XI.th Interdisciplinary Doctoral Conference (IDK), Talk, Hungary, Pécs, 2022.11. 25-26.

Farkas S, Szabó A, Török B, Fazekas CL, Bánrévi K, Correia P, Chaves T, Zelena D: *Effect of gonadectomy-induced hormone deprivation on the progress of Alzheimer's disorder in 3xTg-AD mice,* The International Congress of Neuroendocrinology, ICN2022, Poster, UK, Scotland, Glasgow, 2022.08.7-10.

Farkas S, Szabó A, Kovács T, Ábrahám IM and Zelena D: *17β-estradiol and estrogen-like compound shows neuroprotective potential in a triple transgenic mouse model of Alzheimer's disorder,* FENS Forum 2022, Poster, France, Paris, 2022. 07.09-13.

Farkas S, Szabó A, Kövesdi E, Faludi P, Ábrahám IM, Kovács T and Zelena D: *New estrogen-like compounds with neuroprotective potential in an Aβ1-42-induced neurotoxicity model of Alzheimer's disorder,* School of Neuroscience: from cellular mechanisms to disease modelling, Talk, Italy, Lake Como, 2022.05.09-13.

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